Title

Variable geographic distribution of *Blastocystis* subtypes and its potential implications

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Abstract

Blastocystis is a common intestinal micro-eukaryote found in both humans and non-human hosts and known to be genetically very diverse. It has been divided into numerous subtypes (STs), nine of which have been identified in humans to date. Surveys of ST prevalence have started to emerge over the past few years but to date no data are available for any African country except Egypt and Tanzania. In this study, we determined the prevalence of *Blastocystis* STs in populations from Libya, Liberia and Nigeria, as well as expanding the dataset available for the UK. A total of 356 *Blastocystis* STs were identified in this study, 271 from the UK, 38 from Libya, 25 from Liberia and 22 from Nigeria. SSU rRNA gene sequences revealed the presence of eight of the nine STs known from humans but at varying frequencies between countries. ST1 was the most common ST in Libya and Nigeria whereas ST3 showed the highest frequency in the other two countries, as indeed is the case in most populations around the world. ST4 was absent in Libya and ST2 in Nigeria, while no ST5, ST6, ST8 or ST9 infections were detected in any of the three African populations. The picture emerging from this and other surveys suggests that there is significant variation in ST prevalence between populations. Some of the possible reasons for and implications of this diversity are discussed.

Keywords: *Blastocystis*, diversity, epidemiology

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1. Introduction

Blastocystis is a common intestinal parasitic micro-eukaryote with a worldwide distribution and is often the most frequently detected parasite in epidemiological surveys (Clark et al., in press). Its prevalence varies from country to country and among various communities within the same country. Generally, however, the prevalence in developing countries is higher than in developed countries, which has been linked to standards of hygiene, waste disposal, exposure to animals, and consumption of contaminated food or water (Tan, 2008), although direct evidence for some of these is lacking.

DNA-based methods have been developed that are able to identify genetic variation between *Blastocystis* organisms that are morphologically indistinguishable under the microscope. Molecular studies have focused mostly on determining the prevalence of *Blastocystis* subtypes (STs) in asymptomatic and symptomatic individuals (Böhm-Gloning et al., 1997; Kaneda et al., 2001; Souppart et al., 2009; Stensvold et al., 2011a; Stensvold et al., 2009; Yan et al., 2006; Yoshikawa et al., 2004). In spite of recent methodological advances, the molecular epidemiology of *Blastocystis* infections is still unknown in many parts of the world. Recent studies are starting to provide more information on the distribution of STs among human populations (Tan, 2008; Clark et al., in press) but most have been carried out in temperate regions. To date there is some indication of geographic variation in the prevalence of STs (eg. Forsell et al., 2012a,b) as well as reports of associations between specific STs and disease, although with conflicting conclusions (eg. Domínguez-Márquez et al., 2009; Jones et al., 2009; Stensvold et al., 2011a; Tan et al., 2008).

In this study our aim is to investigate the distribution of *Blastocystis* STs from unselected individuals in North and West Africa, where climates, cultures and ecological conditions are

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likely to be quite different from most regions surveyed to date. This will fill a geographic gap in our knowledge of *Blastocystis* diversity around the world and also provide an insight into whether such variables influence ST distribution. In addition, we greatly expand the data available on ST prevalence for the UK.

2. Materials and Methods

2.1. Source of specimens

UK samples: A total of 271 new *Blastocystis* isolates from samples submitted for routine ova and parasite analysis were studied; 136 came from Irritable Bowel Syndrome (IBS) clinics in England, while the other 135 were submitted by the patient's physician, usually because the patient was symptomatic but the cause was unknown. Both sample groups were received through the Diagnostic Parasitology Laboratory of the London School of Hygiene and Tropical Medicine. The final patient diagnosis is not known and it is likely that some IBS clinic samples were from patients later diagnosed with non-IBS causes of their symptoms and, equally, some of the physician-submitted samples were likely from patients with IBS. Ethical approval for work on the UK samples was obtained (LSHTM reference no.5026).

Libya samples: 150 faecal samples submitted for ova and parasite examination by Libyan patients attending the Sebha Central Medical Laboratory were studied. The samples were submitted in order for the patient to get a health certificate, join the army, or attend university, rather than because the patient was symptomatic. 42 (28%) of these samples were positive by direct smear and 38 of these grew in culture. The Ethics Committee of Sebha University concluded that no specific approval was needed for the further analysis reported here. Sebha is a city in southwestern Libya (27°2'0" North, 14°26'0" East) with a population of 130,000. It was

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historically the capital of the Fezzan region and is now capital of the Sebha District. The weather

is hot and dry in summer and cold and dry in winter.

Liberian samples: 43 faecal samples from children aged 6-18 from Bomi, Bong and Margibi

counties, Liberia, were included. These three counties are to the Northwest, Northeast and East

of the capital Monrovia (6°18′48″North 10°48′5″West), respectively. 30 (70%) of the samples

were positive for *Blastocystis* by PCR. Informed consent was obtained from the parents or school

director for analysis of samples from under-age children. Liberia has a hot equatorial climate

with the rainy season between May to October.

Nigerian samples: 47 faecal samples were obtained from Nigerian patients (median age 32 y,

inter-quartile range: 24-41 y) attending a clinic in Lagos. The Ethics and Experimentation

Committee of the College of Medicine, University of Lagos PEPFAR/APIN and Lagos

University Teaching Hospital, concluded that no ethical permission was needed for this work. 23

(49%) of the samples were positive for *Blastocystis* by PCR. Lagos is a metropolitan city located

in the southwest region of Nigeria (6°27′11″ North, 3°23′45″ East). A typical tropical rainforest

climate exists in the city. The population of Lagos is estimated at over 10 million.

2.2. Sample processing

Approximately 50 mg of each Libyan and UK faecal sample was inoculated into Robinson's

medium (Clark and Diamond, 2002) or (in Libya only) a modification of Jones' medium

(Leelayoova et al., 2002) using a Luria agar slant and supplemented with 10% human serum. The

culture was incubated at 37 °C and examined every two days. Positive cultures were passaged

into fresh medium for another 3-4 days, then *Blastocystis* was harvested by centrifugation and

the pellet resuspended in lysis buffer for DNA extraction using the Puregene core kit A (Qiagen,

Crawley, UK) according to the manufacturer's protocol. This rapid DNA extraction prevents the

possibility of differential ST growth affecting subsequent analyses in our experience. DNA of

samples from Nigeria and Liberia was extracted directly from faeces using the QIAamp DNA

Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

2.3. PCR amplification and sequencing

PCR amplification of partial *Blastocystis* small subunit ribosomal RNA genes (SSU-rDNA) for

subtyping of samples from the UK, Liberia and Libya was performed (at LSHTM) in a 40µl

volume reaction using Biomix (Bioline, London, UK) and the primers BhRDr (5'GAG CTT TTT

AAC TGC AAC AAC G3') and RD5 (5'ATC TGG TTG ATC CTG CCA GTA3') at 2 µM

concentration. These primers amplify a 600 bp region of the SSU-rDNA that contains sufficient

information for unambiguous assignment of STs to samples (Scicluna et al., 2006). The

following amplification profile was used: 30 cycles of denaturation at 94 °C for 30 seconds,

annealing at 59 °C for 30 seconds and extension at 72 °C for 30 seconds. PCR products were

purified using the QiaQuick gel extraction kit (Qiagen, Hilden, Germany) or the GeneJet PCR

Purification kit (Fermentas, Epsom, UK) and sequenced using the BhRDr primer as described

previously (Scicluna et al., 2006).

Nigerian samples were screened for *Blastocystis* by PCR (at the SSI) using the primers

bl1400ForC and bl1710RevC, which amplify a 310 bp SSU-rDNA product, or the primers F1

and BHCRseq3, amplifying a 550 bp product, and Extract-N-Amp PCR ReadyMix (Sigma–

Aldrich Denmark, Brøndby, Denmark). PCR products were sequenced as described previously

(Stensvold et al., 2007; Stensvold et al., 2006).

STs were assigned based on the results of BLAST analysis against the databases at NCBI and/or

www.pubmlst.org/blastocystis. Nucleotide sequences of samples from which unambiguous

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barcode sequences were obtained have been submitted to the isolate database at

www.pubmlst.org/blastocystis.

3. Results and discussion

3.1. UK samples

The samples from the UK had two distinct origins and could therefore have had distinct ST

characteristics: half of the samples were submitted by IBS clinics. The ST profiles of the two sets

of samples were compared using the chi-square test. Although there was a clear excess of ST4 in

IBS samples, the distribution of STs in the two sample groups did not show any significant

difference overall ($p = 0.18$; Table1), so we have combined the two sets of data for geographic

comparisons.

The distribution of STs in the UK samples is generally similar to those reported previously in the

UK and elsewhere in Europe (Table 2), where ST3 is the most frequently detected ST, followed

by ST4, with STs 1 and 2 each having a prevalence of about 10% .

3.2. African samples and comparison with UK samples

In Libya the prevalence of *Blastocystis* has been reported previously to be 26.6% (Alfellani et al.,

2007), which is very similar to the prevalence in the current samples (28%). The prevalence of

Blastocystis in Liberia has not been reported to date and the only report from Nigeria is a

prevalence of 2.5% (in Ogun State) using microscopy (Reinthaler et al., 1988).

The only previous reports of ST prevalence in Africa are from three studies conducted in Egypt

and a small one in Tanzania (Table 2). Libya, Nigeria, Liberia and Egypt cover a range of

distinct climate conditions. Despite this, ST3 was highly prevalent in all countries (ca. 30-60%;

Table 3), although ST1 was detected at the highest frequency in both Libya and Nigeria, with 50% and 45.5% respectively. In neither the Nigerian samples nor in two of the studies from Egypt (Egypt1 and Egypt3; Table 3) was ST2 detected, in contrast to most previous surveys and the other three locations sampled in this study.

Despite ST4 being the second most common ST detected in the UK and being quite common in both Liberia and Nigeria, in Libya and Egypt it was not detected at all. ST6 and ST7 were both found in the UK and ST7 in Libya although rarely. However in Egypt these two STs show a high prevalence in two of the published studies (10-25% for each ST in Hussein et al. (2008) and Fouad et al. (2011)) despite not being found at all in the other study (Souppart et al, 2010). ST5 and ST8 were not detected in any African country but this is perhaps not surprising as they are rare in all human surveys (<1%).

The UK distribution showed a significant difference from all the African studies except that in Egypt by Souppart et al. (2010), although for the Liberian study the difference, while still present, was less striking (0.05> P >0.01). The Libyan and Liberian distributions showed no significant difference from the Nigerian or the Egypt2 (Souppart et al., 2010) distributions, although they differed from each other. It is notable that Souppart et al. (2010) differed from the other Egyptian studies in using SSU-rDNA sequence-based identification of STs, compared to the Sequence Tagged Site (STS) PCR-only approach in the other two. Whether this difference in methodological approach has affected the results of the Egyptian surveys is unclear.

3.3. Review of Blastocystis *subtypes in IBS patients*

Although we found no significant difference between the UK samples submitted by IBS clinics and those submitted from other sources, primarily personal physicians, we feel that some discussion of ST distribution in IBS patients is worthwhile. There have been three previous

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studies carried out on the distribution of *Blastocystis* STs in such patients, in Pakistan, Turkey and Egypt. Since the number of IBS samples in Turkey (Dogruman-Al et al., 2009a) was only five we have limited the comparison with our IBS clinic sample results to the data from Pakistan (Yakoob et al., 2010) and Egypt (Fouad et al., 2011) (Table 4).

In Pakistan, ST1 is the most common ST detected in IBS samples, whereas ST3 is the most frequent ST in Egypt and the UK. ST4 is the second most frequently detected in the UK (37.5%) and is 1.5x more common in IBS compared to other samples (see above). In contrast, ST4 in Pakistan makes up less than 5% of IBS samples and in Egypt it was not detected at all.

The non-IBS ('control') *Blastocystis* STs were also compared from the same studies (Table 5). This time, in all three countries ST3 was the most common ST. Again, in the UK ST4 was the second most frequently detected, but in Pakistan ST1 and in Egypt ST6 were the second most common STs. In Egypt, ST6 and ST7 were both much more common than in either of the other two countries, although even in Pakistan they were more common than in the UK. It is clear that no consistent pattern exists between IBS-linked samples and the ST of *Blastocystis* detected since the distribution observed is very different in each country.

3.4 Aspects of the relative global distribution of Blastocystis *subtypes*

Information on variation in the distribution of STs between geographic locations is only starting to emerge and large gaps remain to be filled on the world map. In this study we have partially filled one gap, in North and West Africa, by presenting data from Libya, Liberia and Nigeria. These data can now be compared to those from other regions of the world.

The difference in the relative diversity and prevalence of STs isolated from humans might reflect different epidemiological and demographic characteristics including climate, geography, cultural habits, exposure to reservoir hosts, and mode of transmission. The ST distribution in the UK

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population sampled in the present study was quite similar to that found in previous UK studies and those from elsewhere in Europe (Table 2). In contrast, ST distributions in the African countries showed variable similarity to each other and to studies performed elsewhere in the world.

The three studies carried out in Egypt used different techniques to identify *Blastocystis* STs. Hussein et al. (2008) and Fouad et al. (2011) used the STS technique and found similar *Blastocystis* ST frequencies (ST1, ST3, ST6 and ST7) even though samples came from different cities in Egypt (Cairo and Ismailia). In contrast, Souppart et al. (2010), also sampling in Cairo, used sequencing and reported ST distributions quite different from the other two studies (Table 3). It is not clear whether the choice of methodology has affected the results in any way, but it suggests that a comparative study is needed using both techniques on the same Egyptian samples. Although Libya has a long border with Egypt and people frequently migrate between the two countries, different *Blastocystis* ST distributions are still observed between the two populations sampled using sequencing methodology (p=0.026).

ST1 has been suggested by some authors to be linked to zoonotic transmission from farm animals (Noël et al., 2005; Tan, 2008). The finding that this ST was the most common in the Libyan population might suggest that exposure to animal faeces could be a significant source of infection. Many people in Libya keep animals at home, including goats and sheep, or on a family farm, such as cows and camels, and they use them for food and milk. However, there is, in fact, a low prevalence of ST1 in domestic animals surveyed to date in Libya (Alfellani et al., in preparation) so it seems unlikely they are a significant source of infection for humans. In contrast ST5 is very common in Libyan domestic animals but was not detected in any human samples from that country.

ST2 also shows evidence of some geographic variation in frequency of detection (Figure 1; Table 2). In Africa, Australia and East Asia it is rare, with a detection rate of 7% or less compared to over 12% in the other regions with reasonable levels of sampling. Once again, however, studies using STS appear less likely to detect ST2 than sequence-based surveys in the same regions (Table 2).

ST3 is the most prevalent overall in humans (44.1%; Table 2) and is the most commonly detected ST in a majority of individual studies. The exceptions are those studies performed in Libya, Nigeria, Thailand, Brazil, and one study each from Pakistan, Iran, and Turkey, where ST1 was the most common; the other exceptions are the studies from Ireland (ST2), Spain (ST4), one study from Denmark (ST4) and one study from Nepal (ST7). The study from Spain was remarkable, since ST3 was not found at all; however, the majority of the patients had acute diarrhoea, and ST4 was similarly found in more than 70% of *Blastocystis* infected patients with acute diarrhoea in one Danish study (Stensvold et al., 2011).

ST4 is the second most common ST in the UK and is commonly found across Europe (Table 2). It is not known why ST4 is so common in Europe and much less frequently detected or absent in the Far East, South America and North Africa (Table 2). Rodents have been identified as a reservoir host of ST4 (Noël et al., 2005; Noël et al., 2003; Silberman et al., 1996; Yoshikawa et al., 1998) but exposure to rodent faeces is likely to be almost universal. It is notable that ST4 is rare in those studies where ST1 is the most commonly identified and that many studies where ST4 is rare or absent were conducted in countries that are subtropical and/or have mostly Muslim populations. However, no information on *Blastocystis* STs is available from other North African countries, many Middle Eastern countries or India, which means that gaps exist in our knowledge of the distribution of *Blastocystis* STs that might allow us to link studies where ST4 is absent across a large geographical area. It is important to remember that our African ST data

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are limited in both sample number and geography. Only one location was sampled in Libya and Nigeria so, for example, we do not know whether the findings from Sebha and Lagos are representative of the whole of Libya and Nigeria or whether we would obtain different results in different parts of these countries, as in the three studies from Egypt (Table 3).

3.5 Correlation between Blastocystis *subtypes and disease*

Whether *Blastocystis* has a role in human disease is uncertain because the organism is found commonly in both symptomatic and asymptomatic individuals (Clark et al., in press). Although many reports have linked *Blastocystis* to gastrointestinal disorders, the clinical importance of this organism has not yet been defined. The parasite has been linked with gastrointestinal symptoms such as watery and mucousy diarrhoea, vomiting, abdominal cramps and bloating. Some epidemiological studies have also suggested a role in IBS (Stark et al., 2007; Ustun and Turgay, 2006). Since *Blastocystis* infection is more common in IBS patients (Jimenez-Gonzalez et al., 2012; Stensvold et al., 2009; Yakoob et al., 2004) and because symptoms linked to *Blastocystis* resemble IBS, the possible role of *Blastocystis* in this syndrome needs to be investigated.

We studied the frequency of STs of *Blastocystis* in patient samples submitted from IBS clinics with samples from other patients in the UK and compared these results with published data from other countries. No consistent link between ST and IBS could be identified across studies in three different countries even though in each case a difference was noted between IBS and non-IBS samples. This does not mean that *Blastocystis* has no importance in IBS, just that any role appears not to be linked to a specific ST.

A difficulty with these studies is the suitability of the control groups used for comparison. In the UK, the samples were all from a diagnostic laboratory and the vast majority of these will be from individuals with gastrointestinal symptoms. There was a higher prevalence of ST4 in the samples

from IBS clinics in the UK although it did not reach statistical significance. It is interesting that ST4 has been linked to non-IBS symptomatic infections in two other European studies (in Spain and Denmark, mentioned above). However, a link between IBS and ST4 is not seen in Pakistan or Egypt, where ST1 is significantly more common in IBS patients than controls.

It was noted earlier that studies in Egypt gave very different results depending on the subtyping methodology used. It is possible that methodology could also be impacting on the IBS results since the IBS studies in Egypt and Pakistan used STS typing while that in the UK used sequencing to assign STs.

4. Conclusion

We have reinforced the emerging picture of substantial geographic variation in *Blastocystis* ST distribution. The reasons underlying this variation are as yet unclear as in many studies little demographic information has been gathered that might yield insights. In the future, sample numbers for new surveys need to be greater than in the three pilot studies reported here, with sampling in multiple geographically and climatically distinct sites where possible. Ideally, future studies would also gather information on the individual's occupation and exposure to animals in order to improve our understanding of the frequently postulated but unproven role of zoonotic transmission. To eliminate uncertainty over the influence of methodology on ST results we recommend the use of sequencing for assigning STs, despite this method's inability to identify STs in mixed infections (Stensvold,in press).

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Figure legend.

Figure 1. Relative distribution of *Blastocystis* STs across various major geographic regions. The

geographic summary data listed in Table 2 are depicted graphically as a percentage of total

samples subtyped for each region.

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Figure 1
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Blastocystis subtype prevalence by geographic region
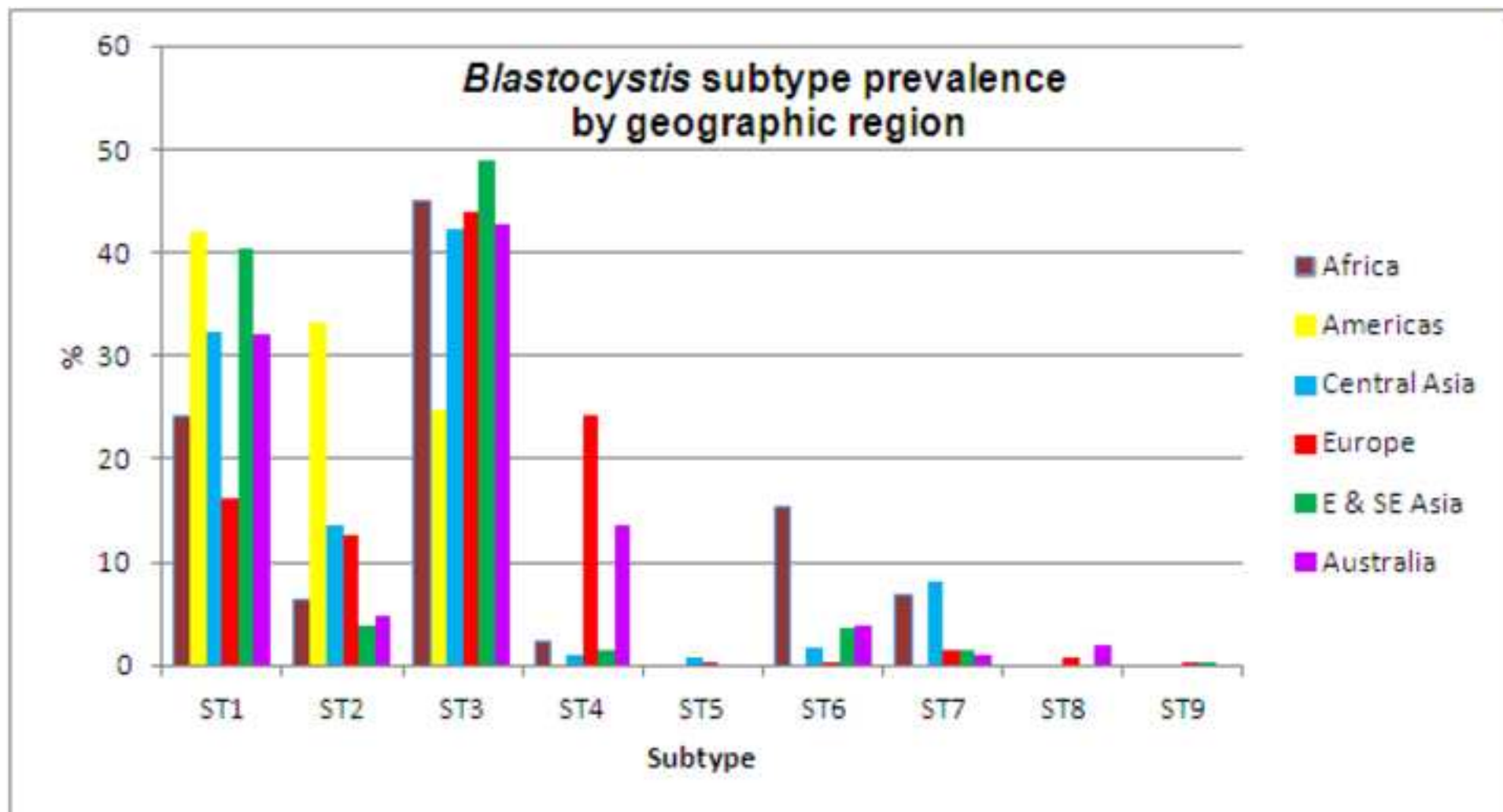
Table 1

Host	Subtype								Total
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	
IBS	14 (10.3 %)	10 (7.4 %)	56 (41.2 %)	51 (37.5 %)	-	1 (0.7 %)	1 (0.7 %)	3 (2.2 %)	136
Unselected	20 (14.8 %)	16 (11.9 %)	58 (42.9 %)	34 (25.2 %)	2 (1.5 %)	-	3 (2.2 %)	2 (1.5 %)	135
Total	34 (12.5%)	26 (9.6%)	114 (42.1%)	85 (31.4%)	2 (0.7%)	1 (0.4%)	4 (1.5%)	5 (1.8%)	271

Table 1. Comparison of *Blastocystis* subtype distribution in IBS clinic- and physician-submitted sample groups in the UK.

Table 2

Country of sample origin	Technique#	No. of subtype observations	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	Mixed ST	Unknown	Reference
								Subtype distribution						
Brazil	Sequencing	73	33	27	13	—	—	—	—	—	—	7	—	Malheiros et al., 2011
Colombia	Sequencing	13	5	4	4	—	—	—	—	—	—	1	—	Santín et al., 2011
USA	Sequencing	7	1	—	6	—	—	—	—	—	—	—	—	Jones et al., 2009
AMERICAS TOTAL		**93**	**39**	**31**	**23**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	
Denmark	Sequencing	29	1	6	15	7	—	—	—	—	—	—	—	Stensvold et al., 2006
Denmark	Sequencing	28	5	9	13	1	—	—	—	—	—	—	—	Stensvold et al., 2007
Denmark	Sequencing	92	20	15	39	16	—	1	—	1	—	7	—	Rene et al., 2009
Denmark	Sequencing	143	31	39	42	24	—	—	6	—	1	26	—	Stensvold et al., 2009
Denmark	Sequencing	25	1	4	1	19	—	—	—	—	—	—	—	Stensvold et al., 2011a
Denmark	Sequencing	24	11	—	13	—	—	—	—	—	—	2	—	Stensvold et al., 2011b
France	Sequencing	43	11	4	23	4	—	—	1	—	—	3	—	Souppart et al., 2009
France	Sequencing	27	1	1	4	17	—	1	3	—	—	—	—	Poirier et al., 2011
Germany	RFLP	171	40	1	118	12	—	—	—	—	—	8	—	Böhm-Gloning et al., 1997
Germany	STS	12	3	2	5	2	—	—	—	—	—	—	—	Yoshikawa et al., 2004
Greece	SSCP	45	9	6	27	1	—	1	1	—	—	—	—	Menounos et al., 2008
Ireland	Sequencing	14	1	6	4	3	—	—	—	—	—	—	—	Scanlan and Marchesi, 2008
Italy	Sequencing	34	3	7	16	6	—	—	1	1	—	4	—	Meloni et al., 2011
Spain	RFLP	51	1	2	—	48	—	—	—	—	—	—	—	Domínguez-Márquez et al., 2009
Sweden	Sequencing	63	10	9	30	13	—	—	1	—	—	—	—	Forsell et al., 2012a&b
UK	RFLP	29	2	1	22	4	—	—	—	—	—	—	—	Clark, 1997
UK	Sequencing	48	2	8	20	16	—	—	1	1	—	1	—	Scicluna et al., 2006
UK	Sequencing	271	34	26	114	85	2	1	4	5	—	—	—	Present study
EUROPE TOTAL		**1149**	**186**	**146**	**506**	**278**	**2**	**4**	**18**	**8**	**1**	**51**	**0**	
Egypt	STS	110	15	—	49	—	—	33	13	—	—	10	—	Fouad et al., 2011
Egypt	STS	44	8	—	24	—	—	8	4	—	—	—	—	Hussein et al., 2008
Egypt	Sequencing	21	4	4	13	—	—	—	—	—	—	1	—	Souppart et al., 2010
Liberia	Sequencing	25	7	7	8	3	—	—	—	—	—	5	—	Present study
Libya	Sequencing	38	19	3	15	—	—	—	1	—	—	—	—	Present study
Nigeria	Sequencing	22	10	—	9	3	—	—	—	—	—	1	—	Present study
Tanzania	Sequencing	6	1	3	2	—	—	—	—	—	—	—	—	Petrášová et al., 2011
AFRICA TOTAL		**266**	**64**	**17**	**120**	**6**	**0**	**41**	**18**	**0**	**0**	**17**	**0**	
Bangladesh	STS	26	2	—	24	—	—	—	—	—	—	—	—	Yoshikawa et al., 2004
Iran	RFLP	40	20	4	16	—	—	—	—	—	—	—	5	Motazedian et al., 2008
Iran	STS	141	48	33	53	—	—	—	7	—	—	33	—	Moosavi et al., 2012
Nepal	STS	20	4	4	12	—	—	—	—	—	—	—	—	Yoshikawa et al., 2009
Nepal	STS	101	40	—	—	—	—	8	53	—	—	41	8	Lee et al., 2012
Pakistan	STS	10	2	—	7	—	—	1	—	—	—	—	—	Yoshikawa et al., 2004
Pakistan	STS	177	87	10	49	8	7	6	10	—	—	—	2	Yakoob et al., 2010
Turkey	Sequencing	87	8	12	66	1	—	—	—	—	—	—	—	Özyurt et al., 2008
Turkey	STS	96	19	22	55	—	—	—	—	—	—	4	—	Dogruman-Al et al., 2008
Turkey	STS	32	20	3	9	—	—	—	—	—	—	—	—	Eroglu et al., 2009
Turkey	STS	38	3	13	22	—	—	—	—	—	—	3	—	Dogruman-Al et al., 2009a
Turkey	STS	75	19	11	45	—	—	—	—	—	—	9	—	Dogruman-Al et al., 2009b
Turkey	STS	25	9	6	10	—	—	—	—	—	—	—	—	Eroglu and Koltas, 2010
CENTRAL ASIA TOTAL		**868**	**281**	**118**	**368**	**9**	**7**	**15**	**70**	**0**	**0**	**90**	**15**	
China	STS	38	17	—	19	—	—	—	2	—	—	5	1	Yan et al., 2006
China	STS	194	56	11	125	1	—	1	—	—	—	10	8	Li et al., 2007a
China	STS	76	18	1	56	1	—	—	—	—	—	2	3	Li et al., 2007b
Japan	STS	32	1	—	30	—	—	1	—	—	—	—	—	Yoshikawa et al., 2000
Japan	RFLP	61	11	13	30	7	—	—	—	—	—	—	3	Kaneda et al., 2001
Japan	STS	50	4	—	26	2	—	11	5	—	2	—	—	Yoshikawa et al., 2004
Malaysia	STS	20	9	1	10	—	—	—	—	—	—	—	—	Tan et al., 2008
Malaysia	STS	38	5	—	20	—	—	11	2	—	—	—	2	Tan et al., 2009
Philippines	RFLP	10	10	—	—	—	—	—	—	—	—	—	2	Rivera and Tan, 2005
Singapore	RFLP	9	2	—	7	—	—	—	—	—	—	—	—	Wong et al., 2008
Thailand	RFLP	159	144	—	13	—	—	—	2	—	—	6	—	Thathaisong et al., 2003
Thailand	STS	5	2	—	2	—	—	1	—	—	—	1	—	Yoshikawa et al., 2004
E & SE-ASIA TOTAL		**692**	**279**	**26**	**338**	**11**	**0**	**25**	**11**	**0**	**2**	**24**	**19**	
Australia	Sequencing	12	5	—	4	2	—	1	—	—	—	1	—	Nagel et al., 2012
Australia	Sequencing	91	28	5	40	12	—	3	1	2	—	—	—	Roberts et al., 2012
AUSTRALIA TOTAL		**103**	**33**	**5**	**44**	**14**	**0**	**4**	**1**	**2**	**0**	**1**	**0**	
EARTH	**Total**	**3171**	**882**	**343**	**1399**	**318**	**9**	**89**	**118**	**10**	**3**	**191**	**34**	

Table 2. Subtype distribution by country of sample origin.
STS= Sequence-tagged site; RFLP=Restriction fragent length polymorphism; SSCP=Single strand conformation polymorphism

Table 3

Country	Subtype									χ² and Fisher's Exact Test results						Reference
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8		UK	Libya	Liberia	Nigeria	Egypt1	Egypt2	
UK	34 (12.5%)	26 (9.6%)	114 (42.1%)	85 (31.4%)	2 (0.7%)	1 (0.4%)	4 (1.5%)	5 (1.8%)	271							Present study
Libya	19 (50%)	3 (7.9%)	15 (39.5%)	-	-	-	1 (2.6%)	-	38	P= <0.0001						Present study
Liberia	7 (28.0%)	7 (28.0%)	8 (32.0%)	3 (12.0%)	-	-	-	-	25	P= 0.022	P= 0.026					Present study
Nigeria	10 (45.5%)	-	9 (40.9%)	3 (13.6%)	-	-	-	-	22	P=0.005	P= 0.140	P= 0.059				Present study
Egypt 1	8 (18.2%)	-	24 (54.5%)	-	-	8 (18.2%)	4 (9.1%)	-	44	P= <0.0001	P= <0.001	P= <0.0001	P= 0.002			Hussein et al., 2008
Egypt 2	3 (15.8%)	4 (20.1%)	12 (63.2%)	-	-	-	-	-	19	P= 0.140	P= 0.026	P= 0.139	P= 0.012	P= 0.005		Souppart et al., 2010
Egypt 3	15 (16.7%)	-	39 (43.3%)	-	-	23 (25.6%)	13 (14.4%)	-	90	P= <0.0001	P= <0.0001	P= <0.0001	P= <0.0001	P= 0.534	P= <0.0001	Fouad et al., 2011
Total	96	40	221	91	2	32	22	5	509							

Table 3. Comparison of *Blastocystis* subtype distribution in some African countries and the UK. The Tanzanian study (Petrášová et al., 2011) was not included due to small numbers. The subtype distributions were compared using the χ2 or Fisher's Exact Test as appropriate.

Table 4

Country	Subtype										Total	Reference
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	Mixed ST	Unidentified ST		
Pakistan	75 (61%)	6 (4.9%)	23 (18.7%)	6 (4.9%)	3 (2.4%)	3 (2.4%)	5 (4.1%)	–	–	2 (1.6%)	123	Yakoob et al., 2010
Egypt	15 (29.4%)	–	22 (43.1%)	–	–	8 (15.7%)	–	–	6 (11.8%)	–	51	Fouad et al., 2011
UK	14 (10.3%)	10 (7.4%)	56 (41.2%)	51 (37.5%)	–	1 (0.7%)	1 (0.7%)	3 (2.2%)	–	–	136	Present study
Total	104	16	101	57	3	12	6	3	6	2	310	

Table 4. Distribution of *Blastocystis* subtypes in IBS clinic samples from different countries.

Table 5

Country	Subtype								Mixed ST	Unidentified ST	Total	Reference
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8				
Pakistan	12 (21.4%)	4 (7.1%)	26 (46.4%)	2 (3.6%)	4 (7.1%)	3 (5.4%)	5 (8.9%)	–	–	–	56	Yakoob et al., 2010
Egypt	–	–	17 (34.7%)	–	–	15 (30.6%)	13 (26.5%)	–	4 (8.2%)	–	49	Fouad et al., 2011
UK	20 (14.8%)	16 (11.9%)	58 (42.9%)	34 (25.2%)	2 (1.5%)	–	3 (2.2%)	2 (1.5%)	–	–	135	Present study
Total	32	20	101	36	6	18	21	2	4	–	240	

Table 5. Distribution of *Blastocystis* subtypes in control groups from IBS studies in different countries.